I. Brief Summary of Rationale for Protocol

The protocol submitted by the UCLA IRB for consideration by the panel is a sub-study of a larger study looking at thymopoiesis in adolescents with HIV. The sub-study uses deuterium-labeled glucose and/or water to study T-cell kinetics, in combination with an evaluation of thymic mass and T-cell markers (TREC) and lymphocyte typing in the main study. The sub-study will be offered at the six month visit to subjects, and involves a 24 hour infusion of deuterium labeled glucose (while located in the UCLA GCRC), combined with two blood collections (50 milliliters depending on body weight) at approximately one and two weeks after the infusion. If there is inadequate labeling of lymphocytes, the subjects will be given 1 milliliter per kilogram of 70% deuterium labeled water orally three times during the first day (under GCRC observation), followed by the same amount of oral D_2O three times daily for the next four days, and then twice daily for one month. There will be two blood collections (50 milliliters depending on body weight) at two and four weeks after the water ingestion. The main study involves physical examinations, blood sampling and CT scans of the upper chest to provide a semi-quantitative assessment of thymic mass.

Three groups of subjects are involved in the research: (1) HIV-positive adolescents and young adults (ages 13 – 21 years), who were infected with HIV either through perinatal (PI-A) or (2) postnatal (AB-A) exposure; and (3) HIV-negative adolescents and young adults (ages 13 – 21 years) as controls (SN-A). The main study will enroll 60-90 subjects total, divided equally into the three groups. The sub-study will enroll 15-30 subjects, divided equally into the three groups of the parent study.

From the panel discussion, this reviewer was left with the impression that the research is both novel and challenging, and would yield important information that may lead to "better therapeutic strategies" for HIV infection. It is reasonable to assume that there will be developmental changes in the outcome measures to warrant the inclusion of HIV-positive adolescents and adults. In addition, the inclusion of the HIV-negative adolescent control subjects are essential to achieving the scientific aims of the research.

II. Review of UCLA IRB Determination

The UCLA IRB decided that it could not approve the use of minors in the sub-study until reviewed by HHS under 45 CFR 46.407, as reflected in the IRB letter to the PI dated February 27, 2003. The stated reason in the letter to OHRP dated July 22, 2002 was that the "healthy" HIV-negative adolescent control subjects do not have a condition, and thus the research could not be approved under 45 CFR 46.406. Clearly the research does not offer the prospect of direct benefit to subjects for participation in the trial, and thus could not be approved under 45 CFR 46.405. Although the UCLA IRB did not state which of the procedures did not qualify as "minimal risk", the IRB determined that the sub-study as a whole presented greater than minimal risk to subjects (and thus was not approvable under 45 CFR 46.404).

The UCLA IRB did not provide an analysis of the risks of the different procedures included in either the parent study or the sub-study. However, the letter to the PI dated December 17, 2001 indicated that the administration of deuterium labeled glucose over a 24 hour period to healthy children cannot be approved under either 45 CFR 46.404, 46.405 or 46.406. This suggests that such administration does not qualify as minimal risk. However, it is not clear whether the duration of the IV administration (24 hours), the use of deuterium-containing compounds, or some other aspect of the procedure is what led the IRB to determine that the

study presented more than minimal risk. In response to the panel's inquiry, the UCLA IRB specifically eschewed any consideration of the risks of individual procedures, stating: "45 CFR 46 at least implies if not compels the IRB to make a risk determination regarding the collective nature of the research procedures and not necessarily parse out assessments of individual procedures. For example, the regulations do not indicate a "procedure by procedure" risk assessment as necessary to making a risk assessment regarding the proposed research."

45 CFR 46.405 and 406 refer to "an intervention or procedure" as the unit of analysis in the text of the regulations. Although 45 CFR 46.404 refers to the more global term of "research", one must still look at each individual procedure and then the overall risk of the procedures to determine whether the research presents no greater than minimal risk. The National Commission endorsed what has been called a "component analysis of risk" in order to avoid the fallacy of the "package deal." In the 1977 Report and Recommendations on Research Involving Children, the National Commission wrote: "To determine the overall acceptability of the research, the risk and anticipated benefit of activities described in a protocol must be evaluated individually as well as collectively, as is done in clinical practice. …If the research also includes a purely investigative procedure [i.e., no prospect of direct benefit] presenting more than minimal risk, the research should be reviewed under [45 CFR 46.406] with respect to such procedure." (page 7, emphasis added) Further, "if the proposed research includes an intervention or procedure from which the subjects may derive direct benefit, it should also be reviewed under [45 CFR 46.405] with respect to that intervention or procedure."(page 10) Similarly, the National Bioethics Advisory Commission recommended that "each component of a study should be evaluated separately, and its risks should be both reasonable in themselves as well as justified by the potential benefits to society or the participants. Potential benefits from one component of a study should not be used to justify risks posed by a separate component of a study." (National Bioethics Advisory Commission, Ethical and Policy Issues in Research Involving Human Participants. Volume I: Report and Recommendations of the National Bioethics Advisory Commission. Bethesda, Maryland, August 2001, page xvi) As the UCLA protocol does not offer the prospect of direct benefit, it may be less important to consider the risks of individual procedures. However, to analyze and then minimize risks appropriately, an understanding of the risks of individual procedures may be useful. *Finally, it is important to dispel the notion that the regulations require a "collective" assessment of risk. In fact, the precise opposite is true – the regulations require an analysis of the risks of each component of the research, with careful attention paid to the presence or absence of the prospect of direct benefit for each individual procedure.*

As clarified in written communication with the panel, the UCLA IRB determined that the procedures included in the sub-study presented a "minor increase over minimal risk." The UCLA IRB did not consider whether the sub-study could have been approved for those HIV-positive adolescent subjects with a "condition" as the inclusion of minors in the sub-study was withdrawn from consideration by the PI pending resolution of the DHHS consultation (letter dated February 21, 2002).

The main study was approved by the UCLA IRB. One can thus infer that the CT scan was considered "minimal risk" as the main study also includes HIV-negative adolescents as control subjects. The analysis of the CT scan procedure by UCLA Radiological Sciences concluded that the estimated effective radiation dose to the subject would be 4 mSv (400 mrem) per study. This

information was included in the consent form where it was indicated that the radiation dose was equal to 16 months of background radiation (assuming that background radiation is about 300 mrem, see http://www.epa.gov/radiation/students/calculate.html). The panel received confirmation of this information through copies of a memorandum to the PI from Dr. McNitt-Gray of the UCLA Radiological Sciences, dated January 30, 2001.

III. Procedures included in the Protocol (including level of risk)

Within the main study, all of the subjects (including the HIV-negative adolescent controls) will undergo a medical and HIV treatment history, physical examination and blood collection every six months for the first 18 months of the 30 month study (i.e., 4 times). Only HIV-infected subjects who have changed their anti-HIV medications will undergo a physical examination and blood collection at 24 and 30 months, in order to determine the impact of the medication change on the outcome parameters being measured in this study. All of these procedures taken alone and in combination present no more than minimal risk.

All of the subjects (including the HIV-negative adolescent controls) will undergo a CT scan of the upper chest only at the first study visit (of the main study). The CT scan may be repeated at 18 months for HIV-positive adolescent subjects depending on the results of their blood work at 12 months. In addition, HIV-positive adolescent subjects who have had a change in their anti-HIV medication may have a repeat CT scan at either 24 or 30 months. In effect, the CT scan will be repeated for those HIV-positive subjects who may have a change in thymus volume based on a change in their clinical status and/or management. Although the CT scan is not part of the sub-study that was submitted for 407 panel review, the fact that the UCLA IRB felt that the combination of all of the procedures presents greater than minimal risk requires us to evaluate the risks of the CT scan.

The estimate of the effective radiation dose will vary depending on the type of CT scanner used. An independent estimate was obtained by this reviewer using the stated parameters (3 mm collimation, pitch 1, 120 KVp, 250 mAs) for the two CT scanners to be used in the study. For an upper chest CT centered on the thymus, the General Electric CTi Single-detector Helical CT Scanner may deliver a Total Effective Dose of 750 mrem (and a Thymus Equivalent Dose of 2900 mrem) and the Siemens Somatom Sensation 16 Multi-detector Helical CT Scanner a Total Effective Dose of 800 mrem (and a Thymus Equivalent Dose of 2900 mrem). Taking the highest Total Effective Dose estimate of 800 mrem, what are the risks of this level of radiation exposure?

A full review of this controversial question is beyond the scope of this protocol review. The reader is referred to the references for a more complete discussion. Briefly, there is no evidence of any risks, particularly a higher incidence of cancer, associated with radiation exposure of this magnitude. In fact, there is some evidence that low-level radiation exposure may have a beneficial effect.[1] At doses below 10 rems (10,000 mrem), there is no evidence of any risk of cancer induction by radiation exposure.[2] Estimates of the risks of low-level radiation exposure are based on the assumption of a linear no-threshold model, for which there is no empirical support. This reviewer thus concludes that a single CT scan of the upper chest (up to 800 mrem) presents only minimal risk to the HIV-negative adolescent controls, and thus is approvable under

[1] Ernst M, Freed ME and Zametkin, AJ. Health hazards of radiation exposure in the context of brain imaging research: special considerations for children. Journal of Nuclear Medicine 39(4): 689-698, 1998.
[2] Charron M, et al. Letter. Pediatric Radiology 2003 (in press).

45 CFR 46.404. In addition, up to three CT scans (2400 mrem) over two years presents no more than a minor increase over minimal risk to the HIV-positive adolescents, and thus is approvable under 45 CFR 46.406. Could one use an MRI scanner instead of a CT scan and thus avoid any radiation exposure altogether? The MRI scan would take considerably longer than the spiral CT scan, which is only a 2 minute study, and thus would present an inconvenience to the subjects. In addition, it is unclear whether the volumetric measurements of the thymus would be comparable using the two different technologies, rendering comparison with the existing scientific literature difficult (as this has been obtained using CT scans).

One oversight in the protocol which is easily remediable is the exclusion of any subject who believes she may be pregnant. *Prior to performing the CT scan, female subjects should be questioned and excluded if there is any chance of pregnancy. The protocol should outline a communication process that must also protect the confidentiality of the adolescent.* Finally, this reviewer notes approvingly that there is no payment for the CT scan. The presence of a stipend in this case may influence an adolescent to be less than truthful when questioned about potential pregnancy.

The subjects who participate in the sub-study (scheduled between month 6 and 12 of the study) will undergo additional procedures, including a 24 hour intravenous infusion of deuterium-labeled glucose, subsequent blood samples (2), followed by drinking deuterium-labeled water and additional blood samples (2) if there is inadequate labeling of the subject's lymphocytes. The duration of involvement in the sub-study will range from 2 to 6 weeks depending on whether the subject goes on to the deuterium-labeled water phase.

Subjects enrolled on the sub-study can expect to have a maximum of anywhere from 100 to 200 milliliters of blood drawn over the course of 6 weeks (i.e., 42 days). The American Red Cross restricts blood donation to individuals ≥ 17 years of age (based on the ability to give consent) who weigh ≥ 50 kilograms, have a hemoglobin level ≥ 12.5 grams per deciliter, and have not donated within the past 56 days. Usually up to a pint of blood (i.e., 473 milliliters) is removed at the time of donation, which translates to a maximum of about 9.5 milliliters of blood removed per kilogram body weight for a standard blood donation. The lower age requirement is not applicable, as the adolescent and parent(s)/guardian(s) will provide consent (i.e., assent and permission) in this study. Based on the Red Cross standards, one could remove 200 milliliters of blood at one sitting from an individual who weighs as little as 20 kilograms, which is well below the 5^{th} percentile for age for a 13 year old adolescent. According to the PI, the volume of the blood samples are within the UCLA guidelines of no more than 1 milliliter per kilogram body weight per month (verbal communication). Assuming the blood hemoglobin of the HIV-negative or HIV-positive adolescent is ≥ 12.5 grams per deciliter, the volume of blood removed (as well as the frequency of sampling) during the sub-study qualifies as no more than minimal risk. *A reasonable exclusion criteria for blood hemoglobin should be added to the sub-study protocol so that the blood sampling remains no more than minimal risk for the HIV-negative adolescents (e.g., ³ 12.5 grams per deciliter) and no more than a minor increase over minimal risk for the HIV-positive adolescents.*

The risks of the 24 hour IV infusion are related to both the difficulty and duration of the IV placement, and the solution that is being infused. *Assuming the IV is being placed by a skilled clinician, and is being observed intermittently during the 24 hours for any complications, this reviewer considers it reasonable to consider the IV to present no more than minimal risk.*

However, the risks of the IV in this case also require an assessment of the risks of the 24 hour infusion of deuterium-labeled glucose, which depend on the preparation and concentration of the glucose solution. The risks of the infusion of dextrose solution can be minimized by restricting the concentration to no more that 5% dextrose (or 5 grams of dextrose per 100 milliliters of fluid), which is the same as a standard maintenance IV solution. Since the maximum dose of deuterium-labeled glucose is 2 grams per kilogram body weight infused over 24 hours, an infusion of 40 milliliters per kilogram per day (or 1.67 milliliters per kilogram per hour) would be required to deliver 1.39 milligrams per kilogram body weight per minute of dextrose. Although the amount of fluid required to deliver this dose of dextrose would exceed by a tolerable amount the usual maintenance fluids for anyone greater than 60 kilograms, the amount of glucose infused at any given body weight is less than the usual amount of glucose necessary to maintain euglycemia in the absence of hyperinsulinism (about 3-4 milligrams per kilogram per minute). Thus, glucose monitoring at 12 and 24 hours should be sufficient to guard against hyperglycemia in those subjects with normal glucose tolerance. *Subjects with a history of glucose intolerance or hyperglycemia (as determined on the routine metabolic panel drawn quarterly for HIV-positive adolescents on HAART treatment) should be excluded from the sub-study. Provided that the dextrose concentration of the glucose infusion is restricted to no more than 5% dextrose, the aspect of the sub-study can also be considered no more than minimal risk.*

Deuterium is a stable isotope, and thus is not radioactive. Although the IRB letter dated July 22, 2002 refers to a "radioactive" substance, this was subsequently clarified as an error. As stated in the FDA letter dated February 25, 1997, the presence of deuterium in either the glucose or water does not require an IND application. However, the deuterium-containing compounds must be suitable for human administration and thus of pharmaceutical grade. The panel requested and received Certificates of Analysis from Cambridge Isotope Laboratories for Lot PR-13035 of D-Glucose-6,6-D_2 which demonstrated chemical purity, sterility, and lack of endotoxin, and for Lot PR-13559 of Deuterium Oxide (70% D_2O) demonstrating a chemical purity of >98%. *These compounds must be prepared according to the Food and Drug Administration Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients (http://www.fda.gov/cder/guidance/4286fnl.pdf, accessed July 12, 2003), and be suitable for either intravenous or oral administration respectively. As the D-Glucose-6,6-D_2 is being provided as a powder to the UCLA pharmacy, the final preparation should be tested to assure that the IV solution contains no more than 5% dextrose. Finally, this reviewer understands that deuterium has a 3 grams per kilogram body weight dose limit. Although it appears that the amount of deuterium administered in this protocol falls well below this threshold, the sub-study protocol should include a calculation of the grams per kilogram of deuterium to be administered in each aspect of the sub-study and assurances that no subject would exceed the dose limit.*

The risks of administration of the deuterium labeled compounds depends on the quality controls that are in place to assure that they are suitable for human administration. With the above assurances and precautions, this reviewer considers it reasonable for an IRB to determine that the 24 hour administration of a 5% dextrose solution (within the above dosing limits) to an assenting HIV-negative or HIV-positive adolescent to be no more than minimal risk. Importantly, the same procedure in a younger child would be more than minimal risk. This reviewer also notes that a significant number of IRB members (and thus IRBs) may consider the administration of <u>any</u> intravenous substance to present more than minimal risk. This reviewer does not consider the 24 hour infusion of a 5% dextrose solution <u>absent any other IV substance</u>

administration to present more than minimal risk for either the HIV-positive or HIV-negative adolescent subjects.

IV. Comments on Permission and Assent Process (Undue Influence and Coercion)

A modest incentive is provided. HIV-negative control subjects will be paid $20 per visit and HIV-positive subjects will be paid $10 per visit for the main study. The difference is due to the fact that the HIV-positive subjects will be seen in combination with clinical visits for routine care. For the sub-study, the protocol indicates that all subjects will be paid $75 for the overnight visit, $25 for the first blood collection, and $50 for the second blood collection. According to the letter dated February 21, 2002, the compensation plan was modified so that the payments for the sub-study blood samples are the same (i.e., $35 per blood sample). This reviewer does not consider these incentives to present an undue influence.

Recruitment for this study will be through a "high risk" adolescent clinic. According to the PI, all of the parent(s)/guardian(s) of the adolescent subjects will be aware of the adolescent's HIV status prior to being approached for this study. As such, there is no risk of loss of confidentiality based on asking the parent(s)/guardian(s) to provide permission for the adolescent to enroll in the study. *However, the need to exclude pregnant women from undergoing a CT scan raises the possibility that an adolescent's right to confidential treatment with respect to pregnancy (depending on California state law) may be compromised. The PI needs to develop a consent procedure by which this information is obtained, yet the adolescent's right to medical confidentiality is protected.*

The consent forms state that the radiation exposure of the CT scan is the same as a routine chest x-ray. The average dose for a routine chest x-ray is more on the order of 20 mrem (as opposed to 400 mrem). This statement needs to be changed to more accurately reflect the effective radiation dose.

Although the assent/permission forms contain a choice about the future use of biological samples, the information provided is insufficient for the adolescent and/or parent(s)/guardian(s) to make a reasonable choice. Will future research be restricted to study related questions, or HIV related questions? If not, does the subject have the right to request that the investigator obtain additional consent for these studies? Will the samples be stored with personal identifiers? Will these samples be provided with or without identifiers? Will immortal T-cell lines be established from the samples? *In addition, the protocol needs to provide more specific information about the storage, access to and oversight of these samples.*

V. Additional Concerns

The consent forms indicate that subjects can request information either about the general study (which is commendable) or specific information about personal results. Test information can only be provided back to the individual subjects if it has been performed in a CLIA-certified laboratory. *Overall, there is a lack of clarity of the nature of the information that can and will be provided back to the subjects. This needs to be resolved.*

The ability to maintain subject confidentiality in a research context may not follow clinical standards, such as legal protections for disclosure of information. *Depending on a local assessment of the ability of the study records to be protected from subpoena, the investigator may want to obtain a Certificate of Confidentiality* (see http://grants1.nih.gov/grants/policy/coc/).

VI. Application of the Criteria for IRB Approval

Provided that minor changes in the protocol are made in accord with the italicized recommendations (above), the risks to subjects are minimized by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk [45 CFR 46.111(a)(1)]. The selection of subjects is equitable, taking into account the purposes of the research and the setting in which the research will be conducted [45 CFR 46.111(a)(3)]. The risks to subjects are reasonable in relation to the importance of the knowledge that may reasonably be expected to result, as there are no direct benefits to the subjects [45 CFR 46.111(a)(2)]. Nevertheless, when some or all of the subjects are likely to be vulnerable to coercion or undue influence, such as adolescents, ... additional safeguards should be included in the study to protect the rights and welfare of these subjects [45 CFR 46.111(b)], requiring us to apply the additional protections found in 45 CFR 46, Subpart D.

Within the main study, all of the procedures to be performed on the HIV-negative controls (i.e., medical history, physical examination, blood collection and a single CT scan of the upper chest) present no greater than minimal risk and thus could be approved under 45 CFR 46.404. For the HIV-positive subjects who change their anti-HIV medications, the three CT scans over two years present no more than a minor increase over minimal risk and thus is approvable under 45 CFR 46.406. Although the UCLA IRB did not provide a risk assessment of the individual and combined CT scans, this reviewer also considers it reasonable for an IRB to determine that an effective radiation dose of no more than 2400 mrem in three doses spread over a two years presents no greater than minimal risk.

Within the sub-study, *provided that changes in the protocol are made in accord with the italicized recommendations (above),* the volume of blood removed (as well as the frequency of sampling) presents no more than minimal risk. For those HIV-positive adolescent subjects with a blood hemoglobin < 12.5 grams per deciliter, the volume of blood removed presents no more than a minor increase over minimal risk. In addition, with the above assurances and precautions, this reviewer considers the 24 hour intravenous administration of a deuterium-containing 5% dextrose solution or the oral administration of deuterium-containing water to an assenting HIV-negative or HIV-positive adolescent to be no more than minimal risk. As the procedures included in the combined main and sub-study are to be performed at time intervals of between one week (e.g., IV administration and blood draws) and six months (e.g., examinations and blood draws), the combined risk of all of the procedures for the HIV-negative control adolescents present no more than minimal risk and are approvable under 45 CFR 46.404. The combined risk of all of the procedures for the HIV-positive adolescents present no more than a minor increase over minimal risk and are approvable under 45 CFR 46.406.

Provided the above italicized recommendations are met, there are adequate provisions for parental permission and child assent (45 CFR 46.408). In addition, there are adequate provisions for monitoring the data collected to ensure the safety of subjects. (45 CFR 46.111(a)(6)), and to protect subject privacy and to maintain data confidentiality. (45 CFR 46.111(a)(7)).

VII. Final Recommendation

Provided that changes in the protocol and consent procedures are made in accord with the italicized recommendations (above), this reviewer recommends (1) that the inclusion of HIV-negative adolescents as control subjects in both the main study and the sub-study is approvable under 45 CFR 46.404; and (2) that the inclusion of HIV-positive adolescents in both the main

study and the sub-study is approvable under 45 CFR 46.406. It is also reasonable that an IRB would consider the entire study (main and sub-study) approvable under 45 CFR 46.404.